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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/840,182	05/05/2004	Michael D. Cleary	STAN-304	7494

24353 7590 09/06/2007

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EXAMINER	
PANDE, SUCHIRA	
ART UNIT	**PAPER NUMBER**
1637	

MAIL DATE	DELIVERY MODE
09/06/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/840,182	CLEARY ET AL.	
	Examiner	Art Unit	
	Suchira Pande	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 July 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-37* is/are pending in the application.

4a) Of the above claim(s) *8,9,12,14-17,20-22,24-27,29,30 and 32* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-7,10,11,13,18,19,23,28,31 and 33-37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *8/10/2007*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission of amendments to claims filed on July 16, 2007 has been entered.

Claim Status

2. Applicant has amended claim 1; withdrawn claims 8-9, 12, 14-17, , 20-22, 24-27, 29-30, 32; cancelled claims 38-41. Claims 1-7, 10-11, 13, 18-19, 23, 28, 31, 33-37 are currently pending and will be examined in this action.

Response to arguments re 102 (b) rejection of claims 1 and 33 over Veres and Stadtman

3. Applicant has amended claim 1 to require that the cell be contacted with a purine or pyrimidine analog having a reactive moiety not normally present in RNA in said cell. Applicant's arguments, see section where applicant provides evidence that 2-thiouridine taught by Veres and Stadtman is naturally found in bacterial tRNAs, filed July 16, 2007, with respect to claims 1 and 33 have been fully considered and are persuasive. Hence the 102(b) rejection of claims 1 and 33 has been withdrawn.

Response to arguments re 103 (a) rejection of claims over Veres and Stadtman and other appropriate secondary references

4. Claims 1-7, 10-11, 13, 18-19, 23-28 and 31 were rejected under 35 U.S.C. 103(a) as being unpatentable over Veres and Stadtman as applied to claim 1 above in view of Trudeau et al. (2001) and further in view of Rana; Claims 33-35 and 37 were rejected under 35 U.S.C. 103(a) as being unpatentable over Veres and Stadtman (1994), in view of Al-Anouti et al. (January 2003) ; Claim 36 was rejected under 35 U.S.C. 103(a) as being unpatentable over Veres and Stadtman (1994) and Al-Anouti et al. (January 2003) as applied to claims 33-35 and 37 above further in view of Iltzsch and Tankersley (1994).

Since rejections based on primary reference Veres and Stadtman have been withdrawn. Accordingly the 103 (a) rejections of above claims over the respective secondary references is also withdrawn.

Claim Rejections - 35 USC § 112

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-7, 10-11, 13, 18-19, 23, 28, 31, 33-37 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988). *Wands* states at page 1404,

> "Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

Enablement issues

This enablement rejection is based on two fundamental enablement problems with the claims. The first problem applies to all of the claims and involves the claims to use a purine or pyrimidine analog having a reactive moiety not normally present in RNA in claim 1; and a uracil analog having a reactive moiety not normally present in RNA in claims 5 and 33; to label RNA. By recitation of such broad language applicants are claiming the use of any and all purine or pyrimidine analogs as well as uracil analogs. During prosecution it has become clear from applicant's remarks that not all analogs can be used because some analogs may not be taken up by cell, or some thiol containing analogs (where thiol is a reactive moiety not normally present in the cell) are converted by cellular enzymes into forms that do not contain the thiol moiety. Thus

applicant does not teach one of ordinary skill how to use all the claimed (purine, pyrimidine and uracil) analogs in the present invention. This is a scope of enablement problem.

The nature of the invention

The claims are drawn to methods of labeling RNA in cells using phosphoribosyltransferase or nucleoside kinase or phosphorylase or UPRT. The invention is in a class of invention which during prosecution has been shown to be unpredictable by applicants remarks. Applicant has provided arguments indicating why certain analogs of uracil and guanosine are not suitable for use in the present method. Thus applicant admits there is unpredictability associated with the present invention.

The breadth of the claims

The claims are broadly drawn to use of all purine or pyrimidine analogs as well as uracil analogs for labeling RNA using the UPRT, HPRT, etc. This means that the claims encompass and assert a method where any purine or pyrimidine analog containing a reactive moiety if taken up by the cell and if the cell contains the claimed enzymes should be able to label RNA. However the specification, while being enabling for use of "2, 4 dithiouracil as an analog having reactive moiety not normally present in RNA in said cell", does not reasonably provide enablement for "any purine or pyrimidine analog having reactive moiety not normally present in RNA in said cell" as recited in claim 1 . The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims as currently recited.

Quantity of Experimentation

The quantity of experimentation in this area is immense because due to the reasons mentioned above, one of ordinary skill would first have to determine if the purine or pyrimidine analog containing a reactive moiety not normally present in RNA of a cell would be taken up by the cell of interest. Next they would have to determine if the metabolism of this precursor by cellular enzymes would not result in some toxicity to the host cell. Finally they would have to determine if the reactive moiety is indeed present in the RNA once the processed precursor molecule is incorporated into the RNA. The above description indicates that It would require significant study and experimentation to determine all the above for **each** purine or pyrimidine analog and uracil analog containing a reactive moiety not normally present in RNA of the cell. This would be an inventive, unpredictable and difficult undertaking in itself. This would require months of inventive effort, with each of the many intervening steps, upon effective reduction to practice, not providing any guarantee of success in the succeeding steps for each chemical chosen as a potential labeling precursor.

The unpredictability of the art and the state of the prior art

Prior art teaches that it is unpredictable which analogs can be used to label RNA. Melvin et al. (Eur. J. Biochem. 92: 373-379) tested 6 different purine or pyrimidine analogs containing a reactive moiety not normally present in RNA to label RNA. They found only 6 thioguanosine and 4 thiouridine labels RNA and RNA from cells exposed to these two compounds does contain thiol residues (reactive moiety not normally present in RNA of cells). However 6-mercaptopurine ribonucleoside, 8- thigunaosine, 2-

mercaptopyrimidine and 2-thio-cytosine were not suitable for labeling RNA (see page 375 par. 1 where reasons are enumerated for each of these 4 precursors were not useful in labeling RNA.

Working Examples

The only working example provided in the specification relates to use of 2, 4 dithiouracil as an analog having reactive moiety not normally present in RNA in said cell.

Guidance in the Specification.

The specification does mention purine or pyrimidne analogs and uracil analogs but only provides specific guidance on use of 2, 4 dithiouracil only .

Level of Skill in the Art

The level of skill in the art is deemed to be high.

Conclusion

In the instant case, as discussed above, the level of unpredictability in the use of any purine or pyrimidine analog or uracil analog, where there is only specific direction provided by the specification for use of 2, 4 dithiouracil and by Melvin et al. for use of 6 thioguanosine and 4 thiouridine supports a finding of undue experimentation. The specification provides one with no written description or guidance that leads one to a reliable method of labeling RNA using any or all purine or pyrimidine analogs. Further the specification does not provide guidance to overcome art recognized problems in determining which precursor would be useful for the method out of a myriads of purine or pyrimidine precursors that seem to fit the bill i.e. they contain a reactive moiety not

normally present in RNA. Finally, the quantity of experimentation is immense. Thus given the broad claims in an art whose nature is identified as unpredictable, the unpredictability of that art, the large quantity of research required to define these unpredictable variables, the lack of guidance provided in the specification, the presence of working example that enables one to use 2, 4, dithoiuracil and the unpredictability demonstrated by prior art balanced only against the high skill level in the art, the inevitable conclusion is that it would require undue experimentation for one of skill in the art to perform the method of the claim as broadly written.

Applicants state (see page 12 of amendment filed on July 16, 2007) " There is no guarantee that a live cell will efficiently take up a given substrate, let alone that that will be a substrate for subsequent steps in the pathway (i.e., the kinase that takes UMP to UTP or the RNA polymerase that incorporates that into RNA) or be bioavailable in an animal." In view of these remarks by applicant, where unpredictability has been indicated, Examiner looked carefully in the specification to check a person of ordinary skill was enabled to use which particular analog for the purposes of this invention. Par. 0082 of the specification provides a working example that teaches one of ordinary skill how to use 2,4 dithiouracil to label the RNA using the method of the claimed invention. Hence specification has certainly enabled one of ordinary skill to use 2,4 dithiouracil but does not enable one to use any purine or pyrimidine analog as presently claimed.

Claim interpretation

6. Applicant has not provided any specific definition of "tag" or "conjugating" in the specification. Examiner is interpreting "tag" to mean any means that may be used to

bind RNA through said reactive moiety. This broad interpretation is the basis of the 102 (b) rejection that follows where thiol moiety of thiol labeled RNA is used to bind thiolated RNA to mercurated cellulose column. "Conjugating" is being broadly interpreted as any mechanism by which the reactive moiety can be bound to the "tag". This binding mechanism includes both, covalent or non-covalent binding.

For the 103 rejections that follow a more narrow interpretation of "tag" and "conjugating" is being used.

Claim Rejections - 35 USC § 102

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

7. Claims 1, 4 , 31 and 33 are rejected under 35 U.S.C. 102(b) as being anticipated by Melvin et al. (1978) Eur. J. Biochem. 92:373-379 and as evidenced by Woodward et al. (1998) Analytical biochemistry 171:166-172 (both references provided by applicant in IDS).

Regarding claim 1, Melvin et al. teach : A method of biosynthetically labeling RNA in a cell of interest (see title) , the method comprising:

contacting said cell with a purine or pyrimidine analog having a reactive moiety not normally present in RNA in said cell (see page 374 last par. where incubation of cells with 6-thioguanosine and 4 thiouridine is taught—both 6 TG and 4 TG contain thio reactive moiety not normally present in RNA (rRNA and mRNA as both rRNA and mRNA were labeled ie thiolated see page 375 par. 3—5) in said cell), wherein said cell comprises a phosphoribosyltransferase or nucleoside kinase or phosphorylase that can specifically incorporate said purine or pyrimidine analog into the corresponding

nucleotide, and wherein said purine or pyrimidine analog is incorporated into RNA (the fact that both rRNA and mRNA are labeled with thiol moieties indicate that the cells taught by Melvin et al. inherently contains a phosphoribosyltransferase or nucleoside kinase or phosphorylase that can specifically incorporate said purine or pyrimidine analog into the corresponding nucleotide, and wherein said purine or pyrimidine analog is incorporated into RNA) comprising said reactive moiety (see page 375 legend and Fig. 1 where newly synthesized thiol containing RNA is shown to bind to mercurated cellulose column, by this teaching its clear that RNA contains said reactive moiety thiol group) ; Teaching by Woodward et al. evidence the fact that both 4 thiouridine (4 TU) and 6 thoiguanosine (6 TG) are useful for in vivo labeling of cellular RNA and should provide a useful method for studying ---gene expression and for isolating and cloning specific mRNAs from mammalian cells---see whole article)

obtaining RNA comprising said reactive moiety from said cell (by teaching RNA extraction from cultured cells—Fig. 1 legend, Melvin et al. teach obtaining RNA comprising said reactive moiety from said cell);

and conjugating a tag to said reactive moiety (Melvin et al. teach binding the thiolated RNA to mercurated cellulose, Melvin et al. teach conjugating a tag (mercurated cellulose) to said reactive moiety (thio moiety on RNA) .

Regarding claim 33, Melvin et al. teach, A method of biosynthetically labeling RNA in a cell of interest (baby hamster kidney cell line BHK-21 was used a cell of interest), the method comprising: contacting said cell with a uracil analog having a reactive thiol moiety not normally present in RNA (See Fig. 4 on page 377 and read Fig.

legend where 4 thiouridine, 4- TU is not normally present in rRNA and mRNA of these cells is used to label cells such that RNA that gets labeled with thiol moiety. The 4 TU is provided to cells through addition to culture media—Fig. 1), wherein said cell comprises a uracil phosphoribosyltransferase (UPRT) that can convert said uracil analog to the corresponding uridine monophosphate; wherein said uridine analog is incorporated into RNA synthesized by said cell (the fact the RNA obtained from this cell is labeled with thiol moiety indicated that the cell does contain uracil phosphoribosyltransferase (UPRT) that can convert said uracil analog to the corresponding uridine monophosphate; wherein said uridine analog is incorporated into RNA synthesized by said cell) .

Teaching by Woodward et al. evidence the fact that both 4 thiouridine (4 TU) and 6 thoiguanosine (6 TG) are useful for in vivo labeling of cellular RNA and should provide a useful method for studying ---gene expression and for isolating and cloning specific mRNAs from mammalian cells---see whole article where Hamster cell RNA is labeled in vivo with thiol moiety is taught—specially page 168 par. 3). Thus prior art clearly teaches both 6 TG and 4 TU are useful for labeling RNA using hamster cell line and teach conditions for labeling with thio nucleosides for optimal recovery of newly synthesized thio-derivatized RNA---see page 168 par. 6)

Regarding claim 4, Melvin et al. teach wherein said reactive moiety is at least one thiol group (see claim 1 above where 6-thioguanosine (6 TG) and 4-thiouracil (4 TU) containing at least one thiol group is taught).

Regarding claim 31, Melvin et al. teach wherein said purine or pyrimidine analog is provided in the form of a nitrogenous base (both 6-thioguanosine (6 TG) and 4-thiouridine (4 TU) taught above see claim 1 above are nitrogenous bases).

Claim Rejections - 35 USC § 103

8. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

9. Claims 1-2, 5-7, 10-11, 13, 18-19 and 33-34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Melvin et al. (1978) Eur. J. Biochem. 92:373-379 and as evidenced by Woodward et al. (1998) Analytical biochemistry 171:166-172 (both references provided by applicant in IDS) in view of Rana (P.G. Pub 2004/0175732 filed on November 17, 2003 with a priority date of November 15, 2002) as evidenced by

Diamandis and Christopoulos (1991) Clin Chem 37 (5) pp 625-636 9provided by Applicant in IDS).

Regarding claim 1, Melvin et al. teach : A method of biosynthetically labeling RNA in a cell of interest (see title) , the method comprising:

contacting said cell with a purine or pyrimidine analog having a reactive moiety not normally present in RNA in said cell (see page 374 last par. where incubation of cells with 6-thioguanosine and 4 thiouridine is taught—both 6 TG and 4 TG contain thio reactive moiety not normally present in RNA (rRNA and mRNA as both rRNA and mRNA were labeled ie thiolated see page 375 par. 3—5) in said cell), wherein said cell comprises a phosphoribosyltransferase or nucleoside kinase or phosphorylase that can specifically incorporate said purine or pyrimidine analog into the corresponding nucleotide, and wherein said purine or pyrimidine analog is incorporated into RNA (the fact that both rRNA and mRNA are labeled with thiol moieties indicate that the cells taught by Melvin et al. inherently contains a phosphoribosyltransferase or nucleoside kinase or phosphorylase that can specifically incorporate said purine or pyrimidine analog into the corresponding nucleotide, and wherein said purine or pyrimidine analog is incorporated into RNA) comprising said reactive moiety (see page 375 legend and Fig. 1 where newly synthesized thiol containing RNA is shown to bind to mercurated cellulose column, by this teaching its clear that RNA contains said reactive moiety thiol group) ; Teaching by Woodward et al. evidence the fact that both 4 thiouridine (4 TU) and 6 thoiguanosine (6 TG) are useful for in vivo labeling of cellular RNA and should

provide a useful method for studying ---gene expression and for isolating and cloning specific mRNAs from mammalian cells---see whole article)

obtaining RNA comprising said reactive moiety from said cell (by teaching RNA extraction from cultured cells—Fig. 1 legend, Melvin et al. teach obtaining RNA comprising said reactive moiety from said cell);

Regarding claim 33, Melvin et al. teach, A method of biosynthetically labeling RNA in a cell of interest (baby hamster kidney cell line BHK-21 was used a cell of interest), the method comprising: contacting said cell with a uracil analog having a reactive thiol moiety not normally present in RNA (See Fig. 4 on page 377 and read Fig. legend where 4 thiouridine, 4- TU is not normally present in rRNA and mRNA of these cells is used to label cells such that RNA that gets labeled with thiol moiety. The 4 TU is provided to cells through addition to culture media—Fig. 1), wherein said cell comprises a uracil phosphoribosyltransferase (UPRT) that can convert said uracil analog to the corresponding uridine monophosphate; wherein said uridine analog is incorporated into RNA synthesized by said cell (the fact the RNA obtained from this cell is labeled with thiol moiety indicated that the cell does contain uracil phosphoribosyltransferase (UPRT) that can convert said uracil analog to the corresponding uridine monophosphate; wherein said uridine analog is incorporated into RNA synthesized by said cell) .

Teaching by Woodward et al. evidence the fact that both 4 thiouridine (4 TU) and 6 thoiguanosine (6 TG) are useful for in vivo labeling of cellular RNA and should provide a useful method for studying ---gene expression and for isolating and cloning specific

mRNAs from mammalian cells---see whole article where Hamster cell RNA is labeled in vivo with thiol moiety is taught—specially page 168 par. 3). Thus prior art clearly teaches both 6 TG and 4 TU are useful for labeling RNA using hamster cell line and teach conditions for labeling with thio nucleosides for optimal recovery of newly synthesized thio-derivatized RNA---see page 168 par. 6).

Regarding claims 2 and 34, Melvin et al. teach wherein sequences encoding said phosphoribosyltransferase or nucleoside kinase (claim 2) UPRT (claim 34) are operably linked to a promoter that is active or can be activated in said cell. (the fact that thiol residues containing moiety gets incorporated into RNA when 6 thioguanosine and 4 thiouridine are fed to cells inherently indicates that these cells (BHK-21) contain phosphoribosyltransferase or nucleoside kinase or UPRT are operably linked to a promoter that is active or can be activated in said cell because if these promoters were not active or could not be activated then no enzyme would have been made and without the enzymatic activity of these recited enzymes the precursors 6 thioguanosine and 4 thiouridine would not get incorporated into RNA).

Regarding claim 5, Melvin et al. teach, A method of biosynthetically labeling RNA in a cell of interest (baby hamster kidney cell line BHK-21 was used a cell of interest), the method comprising:

contacting said cell with a uracil analog having a reactive thiol moiety not normally present in RNA (See Fig. 4 on page 377 and read Fig. legend where 4 thiouridine, 4- TU is not normally present in rRNA and mRNA of these cells is used to label cells such that RNA that gets labeled with thiol moiety. The 4 TU is provided to

cells through addition to culture media—Fig. 1), wherein said cell comprises a uracil phosphoribosyltransferase (UPRT) that can convert said uracil analog to the corresponding uridine monophosphate; wherein said uridine analog is incorporated into RNA synthesized by said cell (the fact the RNA obtained from this cell is labeled with thiol moiety indicated that the cell does contain uracil phosphoribosyltransferase (UPRT) that can convert said uracil analog to the corresponding uridine monophosphate; wherein said uridine analog is incorporated into RNA synthesized by said cell);

obtaining RNA comprising said reactive moiety from said cell (by teaching RNA extraction from cultured cells—Fig. 1 legend, Melvin et al. teach obtaining RNA comprising said reactive moiety from said cell);

Thus regarding claims 1, and 5 Melvin et al. teach all aspects of both these claims but do not teach *conjugating a tag to said reactive moiety-(claim 1) or conjugating a small molecule binding partner to said thiol moiety—(claim 5)* .

Regarding claim 4, Melvin et al. teach wherein said reactive moiety is at least one thiol group (see claim 1 above where 6-thioguanosine (6 TG) and 4-thiouracil (4 TU) containing at least one thiol group is taught).

Regarding claim 31, Melvin et al. teach wherein said purine or pyrimidine analog is provided in the form of a nitrogenous base (both 6-thioguanosine (6 TG) and 4-thiouridine (4 TU) taught above see claim 1 above are nitrogenous bases).

Regarding claim 1, Rana teaches: *conjugating a tag to said reactive moiety.*(see page 1 par. 0004 where Rana teaches addition of tags such as biotin, psoralen-biotin

and 4-thiobiotin to isolated RNA. See Fig. 8 D and page 6, par. 0065 where Rana teaches biotinylated RNA molecule labeled with 4-thio uridine or 6-thioguanosine. Binding to streptavidin coated beads selectively enriches RNA isolated from cell for RNA molecules containing biotin tag.

Regarding claim 5, Rana teaches: *conjugating a tag to said thiol moiety.*(see page 1 par. 0004 where Rana teaches addition of tags such as biotin, psoralen-biotin and 4-thiobiotin to isolated RNA. See Fig. 8 D and page 6, par. 0065 where Rana teaches biotinylated RNA molecule labeled with 4-thio uridine or 6-thioguanosine. The thiol moiety can be used to bind biotin is evidenced by teachings of Diamandis and Christopoulos (1991) Clin Chem 37 (5) pp 625-636 ---ref provided by applicant in IDS where on page 630 last par. they teach ---- 4 thiouridine containing nucleic acid is taught to be biotinylated by using haloacetamido derivative of biotin e.g. iodoacetyl-LC-biotin that reacts with thiol group on the nucleic acid to result in biotinylated nucleic acid. See page 629 par. 4. where they state " another useful class of biotinylated nucleotides consists of nucleotides that contain S-S within the structure of the linker arm. These "releasable" nucleotide analogs can be used in applications where the nucleic acid needs to be released after it's binding to streptavidin.

Regarding claim 6, Rana teaches wherein *said tag is a small molecule binding partner.* (see page 1, par. 0004 where small molecule binding partner biotin is taught as a tag).

Regarding claim 7, Rana teaches *wherein said tag is biotin.* (see page 1, par. 0004 where small molecule binding partner biotin is taught as a tag)

Regarding claim 10, Rana teaches *method further comprising the step of binding a specific binding partner to said tag.* (see page 6, par. 0065 where the step of binding streptavidin, a specific binding partner to said tag biotin is taught).

Regarding claim 11, Rana teaches *wherein said specific binding partner is conjugated to an insoluble substrate for affinity chromatography, and wherein said biosynthetically labeled RNA is separated from non-labeled RNA.* (see page 6, par. 0065 where use of streptavidin coated beads to separate biotin labeled RNA from total RNA is taught. Here specific binding partner (streptavidin) is conjugated to an insoluble substrate (beads) for affinity chromatography, and wherein said biosynthetically labeled RNA is separated from non-labeled RNA.

Regarding claim 13, Rana teaches *wherein said separated RNA is amplified.* (see page 6, par. 0069 where amplification of RNA by reverse transcription is taught).

Regarding claim 18, Rana teaches wherein *said specific binding partner is conjugated to a detectable label.* (see page 13, par. 0145 where avidin and streptavidin conjugated to different detectable labels magnetic particles, superparamagnetic microspheres are taught. Further Rana teaches custom synthesis of beads when biotin/avidin or biotin/streptavidin system is used. Thereby Rana teaches the specific binding partner could be labeled with any other desired detectable label)

Regarding claim 19, Rana teaches *wherein said detectable label is a fluorochrome, radiolabel, heavy metal label, or enzyme conjugate.* (see page 13, par. 0141 where detectable labels such as fluorochrome such as fluorescein, radiolabel such as ^{32}P etc are taught)

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Rana in the method of labeling RNA taught by Melvin et al. The motivation to do so is provided to one of ordiary skill by teaching of both Melvin et al. and Rana. Melvin et al. teach a method of labeling RNA with thiol groups. This thiolated RNA can be further tagged using the biotinylation techniques taught by Rana.

Rana states " -----biotinylated miRNAs can be made incorporating 4-thio uridine or thymidine or 6-thio guanosine in the miRNA sequence, as illustrated in ---.The 4-thio uridine, 4-thio thymidine or 6-thio guaosine can be incorporated into the RNA sequence of the miRNA according to methods known in the art e.g. as described in --------. Total mRNA is isolated from the cell and streptavidin coated beads added to the extract to enrich for the miRNA target complexes---- (see page 6 par. 0065).

Thus Rana explicitly teaches one of ordinary skill that thiolated RNA produced by incorporation of 4-thio uridine, 4-thio thymidine or 6-thio guaosine is a substrate to which biotin tag can be conjugated. Once thiolated RNA is tagged with biotin now the streptavidin chemistry can be exploited for detection of various downstream products resulting from use of labeled RNA. In view of the teaching by Melvin et al. page 374 par. 5 where DTT based elution of thiolated RNA is taught, one of ordinary skill recognizes that the conjugation of tags to RNA via thiol moiety gives the additional advantage that the final product can be released from the tag using dithiothreitol based /cleavage or elution.

10. Claims 3, 23 and 28 are rejected under 35 U.S.C. 103(a) as being unpatentable over Melvin et al. (1978) in view of Rana (priority date of November 15, 2002) as applied to claims 1 and 2 above further in view of Trudeau et al. (2001) Human Gene Therapy 12:1673-1680.

Regarding claim 2, Melvin et al. and Rana teach the method of claim 1. By teaching when precursors 6 thiogunaosine and 4 thiouracil are fed to cells its taken up by cells and thio residues are incorporated into RNA Melvin et al. teach these (BHK-21) cells ***inherently*** contain sequences encoding said phosphoribosyltransferase or nucleoside kinase (claim 2) that are operably linked to a promoter that is active or can be activated in said cell.

Regarding claim 3, Melvin et al. teach method of claim 2 but do not teach *wherein said sequences encoding said phosphoribosyltransferase or nucleoside kinase are exogenous to the cell of interest.*

Regarding claim 3, Trudeau et al. teaches *wherein said sequences encoding said phosphoribosyltransferase or nucleoside kinase are exogenous to the cell of interest.* (see page 1675 par. 4 where Trudeau et. al. teaches stably transduced cell lines A549, H322 and DA3. Here HGPRT sequences are exogenous (derived from *Trypanosoma brucei*.) to the cells (A549, H322 and DA3) of interest.)

Regarding claim 23, Trudeau et al. teaches *wherein said promoter is constitutively active in said cell of interest.* (see page 1676 Fig. 1 and Fig. Legend for Fig. 1 A, where plasmid pTbiGFP expresses HGPRT gene under control of a

constituively active CMV, cytomegalovirus promoter element. Thus Trudeau et. al. teaches wherein said promoter is constitutively active in said cell of interest).

Regarding claim 28, Trudeau et al. teaches *wherein said sequences encoding said phosphoribosyltransferase or nucleoside kinase are introduced into said cell of interest on a replicable vector.* (see page 1674 par. 6 where E.coli cells containing TbHGPRT gene was overexpressed, here TbHGPRT is a cDNA clone provided by B. Ullman. This TbHGPRT cDNA clone is obviously present on a replicable vector that has an inducible promoter capable of expression in *E.coli* cell. Thus Trudeau et al. teaches wherein said sequences encoding said phosphoribosyltransferase are introduced into said cell of interest on a replicable vector.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Trudeau et al. in the method of labeling RNA taught by Melvin et al. and Rana. The motivation to do so is provided to one of ordinary skill by teachings of art and Trudeau et al.

One of ordinary skill knows that if plasmids containing the gene of interest can be introduced into a cell of interest where the enzyme encoded by the plasmid borne gene is expressed then one has a means of exploiting the enzymatic activity of this exogenous gene in the environment of the new host cells.

Trudeau et al. teaches both kinds of plasmids i.e. plasmids containing HGPRT gene that are constitutively expressed and those that are inducible. By combining the method of Trudeau et al. in the method of Melvin et al. and Rana, one of ordinary skill in

the art now has a means to thiol label RNA from any host transformed cell, conjugate a tag to the thiolated RNA and use the tagged RNA for their desired intent.

11. Claims 34-35 and 37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Melvin et al. (1978) in view of Rana (priority date of November 15, 2002) as applied to claims 33 and 34 above further in view of Al-Anouti et al. (January 2003) Biochemical and Biophysical Research Communications vol. 302: pp. 316-323.

Regarding claim 34, Melvin et al. and Rana teach the method of claim 33, As explained above the cells and experimental system used by Melvin et al. inherently must contain a *UPRT gene that was operably linked to a promoter that is active or can be activated in said cell.*

Regarding claim 34, Al-Anouti et al. **explicitly** teaches *wherein sequences encoding said UPRT are operably linked to a promoter that is active or can be activated in said cell.* (see page 317 par. 4 where no of plasmids encoding UPRT under control of modified promoter of *T. gondii* surface antigen 1(SAG1) and their derivatives are taught. The plasmids containing UPRT gene under control of *T.gondii* promoter and pUC19UPRT plasmids all contain UPRT operably linked to a promoter that is active or can be activated in appropriate Human Foreskin Fibroblasts or *E.coli* cells respectively.)

Regarding claim 35, Al-Anouti et al. teaches *wherein said sequences encoding said UPRT are exogenous to the cell of interest.* (see page 317, par. 4 where pUC19UPRT plasmid is taught. These pUC based plasmids can be propagated in

E.coli. Thus Al-Anouti et. al. teaches UPRT gene from *T.gondii* is exogenous to the cell of interest.)

Regarding claim 37, Al-Anouti et al. teaches *wherein said UPRT is Toxoplasma gondii UPRT or a functional derivative thereof.* (see page 316 title and abstract where *Toxoplasma gondii* uracil phosphoribosyltransferase (TgUPRT) is taught).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Al-Anouti et al. in the method of labeling RNA taught by Melvin et al. and Rana. The motivation to do so is provided to one of ordinary skill by teachings of art and Al-Anouti et al.

One of ordinary skill knows that if plasmids containing the gene of interest can be introduced into a cell of interest where the enzyme encoded by the plasmid borne gene is expressed then one has a means of exploiting the enzymatic activity of this exogenous gene in the environment of the new host cells.

Al-Anouti et al. teaches plasmids containing TgUPRT gene that can be expressed in E.coli as well as Human Foreskin Fibroblasts. Thus providing one of ordinary skill the complete repertoire of bacterial shuttle vector as well as mammalian vector. By combining the method of Al-Anouti et al. in the method of Melvin et al. and Rana, one of ordinary skill in the art has reasonable expectation of success in being able to thiolate newly synthesized RNA from cells containing UPRT enzyme. This provides one of ordinary skill a means to thiol label newly synthesiszed RNA, conjugate a tag to the thiolated RNA and use the tagged RNA for their desired intent.

12. Claim 36 is rejected under 35 U.S.C. 103(a) as being unpatentable Melvin et al. (1978) and Rana (priority date of November 15, 2002) as applied to claim 33 above further in view of Maddry et al. (US pat. 5,561,225 Oct 1, 1996) as evidenced by Chan (US Pat. 6,403,311 B1 filed Aug 13, 1999) and Iltzsch and Tankersley (1994) Biochem Pharm. Vol. 48 (4): 781-792 cited by applicant in IDS .

Regarding claim 36, Melvin et al. (1978) and Rana teaches the method of claim 33 but do not teach uracil analog 2,4 dithiouracil.

Regarding claim 36, Maddry et al. teach 2, 4- dithiouracil as a uracil analog (see col. 4 line 3) along with a whole series of Purine and pyrimidine analogs that are made from nucleosides, which contain sulfonate and sulfonamide linkages.

Regarding 2, 4- dithiouracil one of ordinary skill in the art is taught by art that it is an analog that has the characteristic energy emission pattern of a light emitting compound (as evidenced by Chan US pat. 6,403,311 B1 issued Jun 11, 2002 . See col. 11 lines 32-42).

Regarding claim 36, Iltzsch and Tankersley teach uracil analog 2,4 dithiouracil. (see page 781 abstract where 2,4-dithiouracil a uracil analog containing thiol moiety not normally present in cell is taught as a substrate for *T. gondii* enzyme UPRT).

It would have been prima facie obvious to one of ordinary skill in the art to use uracil analog 2,4 dithiouracil out of the numerous analogs taught by Maddry et al. in the method of Melvin et al. and Rana at the time the invention was made.

The motivation to do so is provided to one of ordinary skill in the art by both the art itself as well as Iltzsch and Tankersley. Art teaches one of ordinary skill that 2,4

dithiouracil has the characteristic energy emission pattern of a light emitting compound so when its incorporate in RNA one can use this property to detect its presence. Further, Iltzsch and Tankersley teach 2,4-dithiouracil a uracil analog containing thiol moiety not normally present in cell is taught as a substrate for *T. gondii* enzyme UPRT. This teaching tells one of ordinary skill that 2,4 dithiouracil could be incorporated into RNA using the UPRT enzyme present in the bacterial cell and thiolated RNA can be isolated from such a cell which in turn can be used to conjugate with desired label using the available thiol group in the RNA as a reactive moiety.

Currently, there is a level of unpredictability in the art regarding which thiol containing purine and pyrimidine analogs can be taken up by the cells and successfully incorporated into RNA. This has been demonstrated to the Examiner by explicit response of the Applicant during the prosecution where a number of purine and pyrimidine analogs (thioguanine and 6-Thiogunaine) cited by Examiner where shown to be unsuitable for use in the method claimed in the instant application. Whereas 6 thio guanosine is explicitly taught by art as shown above to be incorporated into RNA to produce thiolated RNA. To one of ordinary skill it is not clear why thioguanine and 6-thioguanine are not suitable where as 6 thioguanosine is a suitable analog. In other words one of ordinary skill in the art must figure out by trial and error which thiol containing analogs will function in the system claimed. Under such circumstances when one of ordinary skill reads the statement " several selected compounds were evaluated as substrates for T.gondii UPRTase, and it was found that in addition to emimycin and 5-fluorouracil, 2,4-dithiouracil is also a substrate for this enzyme" (see page 781, last 4

lines of Iltzsch and Tankersley). This clear teaching by Iltzsch and Tankersley in view of the other properties associated with 2,4-dithiouracil enunciated above, one of ordinary skill would be motivated to try using 2,4-dithiouracil as a substrate for the present invention in addition to 4 thiouridine taught by Melvin et al.

One of ordinary skill is provided enough reasons by art to try and see if 2,4-dithiouracil when fed to the cells containing UPRTase will be taken up and incorporated into newly synthesized RNA.

Conclusion

13. All claims under consideration 1-7, 10-11, 13, 18-19, 23, 28, 31 and 33-37 are rejected over prior art.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Suchira Pande whose telephone number is 571-272-9052. The examiner can normally be reached on 8:30 am -5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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